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Genetic diversity in a captive population of Asian lions

**Title:** An assessment of the genetic diversity of the founders of the European captive population of Asian lion (*Panthera leo leo*), using microsatellite markers and studbook analysis.

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## Genetic diversity in a captive population of Asian lions

**Abstract**

A European Endangered Species Programme (EEP) was established in the early 1990s, in order to manage a captive population of Asian lions (*Panthera leo leo*) within European zoos. The founders of this population comprised of nine individuals that originated from a captive population in India. During 2007-2009, 57 lions were born in the European captive population. Of these births, 35 individuals died within 20 days, three died within two months and one individual was euthanased at four months old. Indeed, over 50% of the total historical captive population died within 30 days of birth. The 'European Studbook for the Asian Lion' shows that the EEP founder population contains individuals from matings of full and half siblings, including all female founders.

It is probable that high levels of inbreeding within this captive population are causing high levels of stillbirths and infant mortality. Previous research has shown that there is limited genetic variation in the captive population in India. This study uses the same microsatellite markers to establish the level of genetic variation that was present when the EEP population was established in comparison with that observed in the Indian zoo population, from which it was derived. Only three of the 12 microsatellite markers, showing variation in the Indian captive population, showed bi-allelic heterozygosity in the EEP founders, indicating that most variation was not present during the establishment of the EEP population. Therefore, the future of the Asian lion EEP is compromised by lack of genetic variation and high levels of inbreeding, which can only be alleviated by importing further individuals with different genotypes from India.

**Keywords:** Asian lion, captive breeding, genetic variation, microsatellite, *Panthera leo*.

**Introduction**

The financial resources required to maintain captive populations make it essential that the individuals captured from the wild (founders) are sufficient and genetically suitable to allow for the development

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of a viable, long-term population (Goldstein *et al.*, 2000, Goncalves da Silva *et al.*, 2010, Ivy *et al.*, 2009). This is also vital if the species is part of a conservation effort, where individuals bred in captivity may one day be used as stock for reintroductions or to supplement existing wild populations, as the individuals released in the future must be genetically comparable to their wild counterparts to allow the re-establishment or enhancement of viable populations (Frankham, 2010).

However, the effective establishment of captive populations of endangered species relies on balancing many ecological, biological and financial factors (Barnett *et al.*, 2006a, Barnett *et al.*, 2006b, Dubach *et al.*, 2005, Russello and Amato, 2004).

As technology and scientific knowledge advance, the methods available for assessing suitability of founder individuals improve, and involve the integration of many different fields of research (Barnett *et al.*, 2006a, Dubach *et al.*, 2005, Russello and Amato, 2004, Goldstein *et al.*, 2000, Ryder, 1986). Advancements in DNA technology and its application as a conservation tool allow an assessment of genetic variability within wild populations, and the effectiveness of capture of this genetic variation in founders (Russello and Amato, 2004, Gilbert *et al.*, 1991, Goncalves da Silva *et al.*, 2010, Ryder, 1986, Frankham *et al.*, 2010). In short, captive breeding programmes strive to maintain most of the founder genetic diversity over time, typically >90% for >100 years (Goncalves da Silva *et al.*, 2010, Ivy *et al.*, 2009, Russello and Amato, 2004). However, in the case of the Asian lion (*Panthera leo leo*) the establishment of the captive population occurred before the analysis of genetic data was possible, and as such, the population was established without access to, or consideration of, this information (O'Brien *et al.*, 1987, Boakes *et al.*, 2007). Analyses of studbook data are typically used to estimate degrees of relatedness of and levels of inbreeding in descendants of founders, based on the assumption that founders are unrelated to each other. However, this assumption is often violated and hence the viability of many captive breeding programmes is potentially compromised by lack of genetic data.

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Historically, the lion was found extensively across Africa, Europe, the Middle East and Asia up to the middle to late Pleistocene (Antunes *et al.*, 2008, Barnett *et al.*, 2006b). During the global mass extinction of megafauna in the late Pleistocene, the lion's range was severely reduced and it disappeared from Europe around 2,000 years ago, owing to human hunting and habitat loss. The lion's range was further reduced during the 19<sup>th</sup> and 20<sup>th</sup> centuries, when populations were extirpated in the Middle East and North Africa (Antunes *et al.*, 2008, Barnett *et al.*, 2006b, Black *et al.* 2013). A remnant population of lions has been isolated in the Gir Forest, Kathiawar, Northwest India, which is the only surviving wild population of the putative subspecies *Panthera leo persica*. Recent phylogeographical research and a review by the IUCN Cat Specialist Group's Cat Taxonomy Task Force have led to a revision of lion subspecies, so that *Panthera leo persica* is now included in the nominate *Panthera leo leo*, which ranged originally from India through the Middle East and North Africa to West Africa (Bertola *et al.* 2016; Kitchener *et al.* 2017). However, currently isolated and fragmented populations may be recognised as separate management units, including the surviving Asian lions in the Gir Forest and the few surviving West African populations. The option remains for the future, if inbreeding becomes a serious issue, for genetic exchanges between Asian and West African populations. The Asian population is commonly believed to have been reduced to perhaps around 20 individuals by the late 19<sup>th</sup> century. With strict protection, the population has gradually recovered. The most recent census of the Gir Forest population in 2015 reported at least 523 individuals occurring in a wider geographical area, although no assessment of their genetic viability was given (Guardian, 2015).

In the 1970s a captive founder population of Asian Lions was established in India from the wild population. In the early 1990s eight descendants of these founders and one wild-caught individual were transferred from India to three European zoos (Zurich, London and Helsinki) to establish a European Endangered Species Programme (EEP). This population is noteworthy in that the 'genetic' founders of the population, *i.e.* the sole providers of all genetic material to the EEP population ( $n=9$ ),

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possess a documented pedigree history. In December 2009 the EEP population of Asian lions comprised 93 individuals in 34 zoos (Dorman, 2009).

The presence of historical pedigree data for the EEP population allows a more accurate analysis of F-values (the inbreeding coefficient *of* an individual) and mean kinship values (the level of relatedness *among* individuals within a group or population). F-values give a numerical value of between 0 and 1, which is the probability of the inherited copies of DNA being identical by descent, i.e. coming from the same ancestor. The higher the value, the more common ancestors an individual possesses and, therefore, the more inbred it is considered to be. In order to obtain a true representation of genetic variation present when the EEP population was established, any genetic analysis should be restricted to the nine EEP founders.

In wild populations 20% of all Asian lion cubs born reach more than two years old. Cub deaths in the wild are mainly caused by starvation, abandonment and infanticide by adult males following pride takeovers (Kalahari Predator Conservation Trust, 2013). In captivity, where food is plentiful and veterinary care is available, cub mortality should be minimal. However, since the establishment of the EEP population over 50% of offspring have died within 30 days of birth (Dorman, 2009). During 2007-2009, 57 cubs were born in the EEP population; 18 of these cubs died within 24 hours of birth, a further 12 died within five days, and another four individuals survived for 20 days or less (Dorman, 2009). This equates to a total cub mortality of 61.4% in the three weeks following birth, and a total cub mortality rate of 68.4% during the 2007-2009 period. This can be compared to a cub mortality rate of around 40% at Sakkarbaug Zoo in India in the mid-1990s (Ashraf *et al.*, 1993). Whilst the costs and ethical issues involved with transporting animals over long distances may make it more appealing to arrange an exchange or transfer within the EEP region, it is important that the long-term viability of the offspring and the captive population as a whole must be considered (Barnett *et al.*, 2006b, Dubach *et al.*, 2005).

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Genetic variation in the population of Asian lions from Indian zoos has previously been characterised (Gaur *et al.*, 2006, Singh *et al.*, 2002). These studies suggest that historic bottlenecks, caused by range restriction and hunting, did not reduce genetic variability to an irrecoverable level. Importantly, these characterisation data allow a direct comparison of the variability observed in the captive Indian population and the variability present when the EEP population was established.

In this study, levels of genetic variation were established in the nine founders from the EEP population using microsatellite markers. The results were compared with published genetic variability data from the Indian captive population, allowing a direct comparison between the two populations.

### Materials and Methods

#### Extraction

Samples (bone, skin and muscle tissue) from the nine dead Asian lion EEP founder individuals were sourced from museums and zoos within the EEP (Table 1). Tissue extraction was carried out using QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocol (QIAGEN Inc). DNA from bone and museum skin samples was extracted using an in-house decalcification and digestion method prior to QIAGEN DNeasy protocol. 50 mg bone powder or fragmented skin was incubated overnight at room temperature in 1 ml 0.5 M EDTA, followed by a double-distilled water wash (1 ml ddH<sub>2</sub>O). 360 µl ATL, 40 µl Proteinase K (>600 mAU/ ml) and 10 µl 1 M DTT were added to the tube and incubated under agitation at 55 °C for a further 24 h. 400 µl AL was added and incubated at 70 °C for 30 min. 400 µl 100% EtOH was added before completing the extraction with the QIAGEN DNeasy protocol.

#### PCR amplification

Individuals were genotyped using 12 species-specific microsatellite markers (Gaur *et al.*, 2006, Singh *et al.*, 2002). These markers were Ple23, Ple24, Ple51, Ple55, Ple57, Ple21, Ple34, Ple53, Ple58,



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Ple62, Ple65, and Ple251. Products were amplified in 25 µl reactions using illustra™ puReTaq Ready-To-Go PCR beads (GE Healthcare, Uppsala, Sweden), 5 µl template DNA, and 0.4 µM of each primer. The amplification differed in the number of cycles depending on the source DNA; tissue samples were subjected to 30 cycles, whereas bone and museum skin required 35 cycles of PCR. The protocol was as follows: Denaturation at 94 °C for 3 min, 30 or 35 cycles of 94 °C for 30 sec, 58 °C for 20 sec, 72 °C for 1 min, with a final extension of 72 °C for 30 min. PCR amplification was carried out using a Techne TC-5000 thermal cycler. PCR amplification was repeated at least six times for all of the bone and museum skin extracts. Just over 40% of the tissue samples were confirmed by repeated testing (25/60 tests).

### Analysis

Forward primers were 5'-fluorescently tagged for automated genotyping. Alleles were separated using capillary electrophoresis (CE) in the ABI3730 Genetic Analyser (Applied Biosystems, Lincoln, USA) and carried out independently by NERC Biomolecular Analysis Facility (Sheffield, UK). Applied Biosystems PeakScanner Software (v.1.0) was used to establish peak data. Flexibin (Amos *et al.*, 2007) was used to bin alleles. Genotype results were scored following Taberlet *et al.* (1996) (where necessary), and quality was assessed using the scoring technique of Miquel *et al.* (2006).

The studbook was analysed to assess the pedigree relatedness of the nine EEP founders and to ascertain the movements among zoos of the founding lions in the early stages of the studbook. Observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity were calculated using GENETPOP v.4.2 (Raymond and Rousset, 1995, Rousset, 2008), and PMx software (Lacy *et al.*, 2012) was used to calculate F-values.

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**Results****Microsatellite analysis**

Table 2 summarises the microsatellite analysis results in comparison to the previously published Indian population data. Markers Ple23, 51, 53, 62, 65 and 251 amplified a consensus homozygote profile in all individuals tested. Markers Ple21, 34 and 58 all produced a consensus homozygote profile in all successfully genotyped individuals, but for some individuals we could not generate a genotype (specifically, marker Ple34 failed to amplify in individuals 6 and 8, and individual 2 failed to amplify at markers Ple21 and 58)..

Ple55 and Ple57 showed bi-allelic heterozygosity with  $H_o$  figures of 0.778 and 0.334 respectively (Table 2). Individual 8 was scored as a heterozygote at marker Ple55 with 4 out of the 6 repeated tests producing the heterozygote profile; (quality score 0.666).

Marker Ple24 produced a homozygote profile in all individuals, except in individual 8, which was scored as a heterozygote with 2 out of the 7 repeated tests producing the heterozygote profile; (2 out of 7 repeated tests, quality score 0.286). The remaining five amplifications demonstrated drop-out of either one of the alleles. The  $H_o$  was 0.111 for this marker (Table 2).

Owing to some of the failures of repeated tests, the results for individual 8 and individual 2 should be treated with caution. Results from marker Ple58 should also be considered cautiously as, owing to the large amplicon size, the failure of repeated tests was high in three individuals (1, 7 and 8) and failed completely in individual 2. These individuals are the four individuals sourced from bone and museum skin (1, 2, 7 and 8) and, as such, their age and degradation could have adversely affected success rates.

The observed heterozygosity between the EEP founders and the current Indian captive population was compared (Figure 1). In the EEP founders, nine of the 12 markers were monomorphic ( $K=1$ ) in all

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tested individuals. For the remaining three markers, the levels of observed heterozygosity were lower in the EEP founders than in the Indian captive population and were only observed in bi-allelic form ( $K=2$ ).

Using human nucleic acid mutation rates as a guide, it is estimated that 16,000 generations would be required for a mutation to occur in a singular target region of the fragments analysed in this study, and it is unlikely that the excessive homozygosity observed here is due to the phenomenon of allelic drop-out, because homozygosity is observed across multi-locus markers (Dakin and Avise, 2004).

### Studbook data

Using the 'European Studbook for the Asian Lion Number 5' (2007-2009), the pedigrees for the nine EEP founders were established (Figure 2). From the capture of the wild individuals (1972-1990) to the transfer of the EEP founders to European zoos (London, Helsinki and Zurich) in 1990-1992, there were ten matings traced back to seven of the wild-caught founders. These matings produced six of the nine EEP founders (4-9); one EEP founder was sourced directly from the wild-caught population (3) and the remaining two were the offspring of captured pregnant females (1 and 2). It is important to note that these two individuals represent cubs that had not been born at time of capture of the female lions and the sires are unknown. It is possible, but not implied, that the sires of these cubs may already be represented in the pedigree, further compounding the issue. Six of the individuals (4-9 on the schematic) share a second generation common ancestor, their grandfather, Individual 'I'. These six individuals include the entire female EEP founding population ( $N=5$ ), along with one male.

Three individuals are the offspring of full-sibling matings (4-6), and a further two are the offspring of half-sibling matings (7 and 8). These five individuals also represent the entire founding female population transferred during the establishment of the EEP. Females 4-6, as the offspring of full-siblings, have an  $F$ -value of 0.25. Females 7 and 8, as the offspring of half-sibling matings, have an  $F$ -value of 0.125.

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Assuming non-relatedness of the initial wild-caught founders, and the assumption that Individuals 1-3 do not share a pedigree with each other or with individuals 4-9 before being brought into captivity, then three males share no common ancestors with these inbred females. This allowed two zoos to receive unrelated individuals, based on these assumptions. London received a sister pair, who were the offspring of full-sibling parents (5 and 6), while Helsinki received the sister pair offspring from a half-sibling mating (7 and 8). However, both zoos received potentially unrelated males; London received two (1 and 2) and Helsinki one (3). Male 9 was related to all the founder females, sharing two common ancestors with females 7 and 8 (kinship value of 0.0938), and one common ancestor with 4, 5 and 6. He was transferred to Zurich with one of his female relations (4), with whom he shared the sole common ancestor, individual 'I' (kinship value of 0.0625).

### **EEP management**

A review of the current Asian lion studbook reveals examples of questionable breeding management from within the population. For example, two EEP founders at Helsinki produced a total of 13 cubs from four litters. One of these cubs, a female, was transferred via London to Paignton, where she mated with a resident lion (pairwise kinship, 0.0313). These matings produced six litters and 14 cubs. One of these female cubs was transferred in June 2000 to Boissière, where she was joined by her grandfather in May 2001. This pair produced an initial litter of two viable offspring ( $F=0.125$ ). The matings continued and three single-cub litters were born, which did not survive the first few weeks. This female was then transferred to another zoo, whilst pregnant for the fourth time with two more of her grandfather's cubs. Of seven cubs born from these five litters, only three have survived to breeding age.

### **Discussion**

This study has shown that the founding EEP Asian lion population had excessive genetic homozygosity for 12 microsatellite markers. This very low genetic variability in Asian lions could be

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the result of historic bottlenecks or the founder effect in captivity. The Indian captive population shows much more variation and yet endured the same events (Antunes *et al.*, 2008, Barnett *et al.*, 2006b, Burger *et al.*, 2004, Driscoll *et al.*, 2002, O'Brien *et al.*, 1987, O'Brien, 1994, Paulson, 1999). Using identical markers, heterozygosity was observed at all 12 loci in the Indian population (Gaur *et al.*, 2006, Singh *et al.*, 2002), whereas the EEP founders only showed variability at three loci, with only two alleles being observed at each locus (Table 2). These data are not the result of sampling bias, because samples from all founders were available, representing the entire genetic contribution to the EEP population when it was established. Studbook analysis shows that reduced genetic variability in the EEP population is probably caused by inappropriate matings between the original wild-caught individuals from India prior to the establishment of the EEP and incorrect assumptions about the relatedness of founders. The studbook documents matings between full- and half-siblings, which in turn produced inbred offspring that were transferred to the European zoos to establish the EEP population. All founder females and one founder male share the same grandfather. Despite limited opportunities to use genetic information from the founder population at the time, a historic pedigree was available and, therefore, it is disappointing that unrelated founders were not sourced and that subsequent inbreeding was not avoided in the EEP population. It should be noted that early in this study the problem of inbred founders was immediately noted, without the aid of any genetic data.

However, whilst previous knowledge of kinship, or control over the pedigree of the founder lions, can be assumed to be outside the control of the EEP management programme, there is evidence of widespread unsuitable matings since the inception of the EEP population. For example, there is evidence of matings between close relatives (first- and second-order) producing one individual with an F-value of 0.31, and an individual whose grandfather was also her father. In the latter example, the transfer of the grandfather to the same location as his granddaughter led to low viability of the highly inbred cubs. The vast majority of captive breeding programmes use the PMx software (Lacy *et al.*, 2012) to generate relatedness values and provide breeding recommendations. A fundamental assumption of PMx is that the founders of a studbook are unrelated and downstream pairings are

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made accordingly. However, as all captive populations are founded from the wild stock, there will always be some ambiguity regarding relatedness of founders as the wild pedigree is unknown (Russello and Amato, 2004). Both the studbook analysis and the genetic profile provide strong evidence that for the Asian Lion EEP this assumption of unrelatedness is invalid, with the inevitable outcome that all subsequent relatedness values calculated by the studbook would underestimate both relatedness and the threat of inbreeding depression. Previous studies have compared assumed pedigree relatedness against genetic relatedness based on actual data from the same population. These studies have produced a misclassification rate of between 40-80%, where genetic data produced a profile of relatedness which was genealogically incorrect (Gautschi *et al.*, 2003, Ivy *et al.*, 2009, Russello and Amato, 2004). The Asian lion EEP is one of the most high-profile captive animal populations and is seen as a flagship breeding programme. However, current evidence demonstrates both genetic impoverishment as well as a clear breach of the fundamental studbook assumption of founder unrelatedness. Therefore, the results of this study and others suggest it is highly likely that other EEPs are in a similar situation that is as yet undiscovered. It would be beneficial for all EEPs to undergo a genetic screening programme to enable the most effective management and maximise maintenance of genetic diversity. If these programmes are managed with inaccurate relatedness coefficients, it is likely that valuable genetic variation is being lost, thereby potentially compromising captive breeding programmes. Indeed, previous studies have demonstrated that when there are related founders, there is a rapid increase of inbreeding within the first few generations of captivity (Ivy *et al.*, 2009).

For the future of the captive Asian lion population, as with many other species in captive breeding programmes, population managers must make full use of all the tools that are available to minimise inbreeding and promote long-term viability of populations. Information provided by genetic markers and the use of population management software, such as PMx, can give sound recommendations on pairings in captive populations, based on genealogical and genetic data.

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However, given the results provided in this study, demonstrating very low levels of genetic variation and therefore extremely high degrees of genetic similarity between individuals in the Asian lion EEP population, it is highly unlikely that any favourable pairings would be identified. In fact, the majority of matings could be considered detrimental. The data from this study suggest the EEP Asian lion population has poor long-term viability. Recently, new founders have been sourced from the Indian captive population, including Aalborg (one male 2014), Rotterdam (one male 2014), Prague (one male and two females imported October 2016) and London (one male and one female imported December 2016), but we are not aware that these have been screened genetically prior to importation. Although they should have been selected from the studbook for their unrelatedness to animals in Europe, genetic testing could confirm any introduced variability. These newly-imported animals should be prioritised for breeding and their subsequent offspring incorporated into the EEP to maximise outbreeding potential.

## Conclusions

The Asian lion is one of the most endangered felids in the wild with a global population of just over 500 individuals. Hence, it is vital that captive populations are managed appropriately so that they can act as a genuine back-up to wild populations. This study indicated that the Asian lion EEP population contains only a small proportion of the genetic variation found in the Indian captive population at the microsatellite markers studied. However, this is not necessarily indicative of a complete lack of variation within the EEP population, although it is of concern that considerably more variation was observed at the 12 microsatellite markers in the comparable population. Whilst the purpose of this particular research was to directly compare genetic variation between the two captive populations, future research could increase the number of selected loci to ascertain the depth of this homogeneity in the EEP population or attempt to locate variation at a deeper level, e.g. SNPs. We recommend that the EEP population should be supplemented with individuals from the Indian captive population that have been carefully selected based on genetic variation they can bring to the population to increase

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genetic diversity in order to enhance the long-term viability of this important back-up population. It is vital that breeding programmes are robust and benefit endangered species.

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### References

- Amos, W., Hoffman, J. I., Frodsham, A., Zhang, L., Best, S., Hill, A. V. S., 2007. Automated binning of microsatellite alleles: problems and solutions. *Molecular Ecology Notes*, 7, 10-14.
- Antunes, A., Troyer, J.L., Roelke, M.E., Pecon-Sattery, J., Packer, C., Winterbach, C., Winterbach, H., Hemson, G., Frank, L., Stander, P., Sieffert, L., Driciru, M., Funston, P.J., Alexander, K.A., Prager, K.C., Mills, G., Wildt, D., Bush, M., O'Brien, S.J., Johnson, W.E., 2008. The evolutionary dynamics of the lion *Panthera leo* revealed by host and viral population genomics. *PLoS Genetics*, 4, e1000251.
- Ashraf, N. V. K., Chellam, R., Molur, S., Sharma, D., Walker, S., 1995. Asiatic Lion (*Panthera leo persica*). Population and Habitat Viability Assessment and Global Animal Survival Plan Workshops. Baroda, India.



## Genetic diversity in a captive population of Asian lions

Barnett, R., Yamaguchi, N., Barnes, I., Cooper, A., 2006a. Lost populations and preserving genetic diversity in the lion *Panthera leo*: Implications for its ex situ conservation. *Conservation Genetics*, 7, 507-514.

Barnett, R., Yamaguchi, N., Barnes, I., Cooper, A., (2006b. The origin, current diversity and future conservation of the modern lion (*Panthera leo*). *Proceedings of the Royal Society B-Biological Sciences*, 273, 2119-2125.

Bertola, L.D., Jongbloed, H., Van Der Gaag, K.J., De Knijff, P., Yamaguchi, N., Hooghiemstra, H., Bauer, H., Henschel, P., White, P.A., Driscoll, C.A., Tende, T., 2016. Phylogeographic patterns in Africa and High Resolution Delineation of genetic clades in the Lion (*Panthera leo*). *Scientific reports*, 6, 30807.

Black, S.A, Fellous, A., Yamaguchi, N. and Roberts, D.L.. 2013. Examining the extinction of the Barbary lion and its implications for felid conservation. *PLOS One*, 8, e60174.

Boakes, E.H., Wang, J., Amos, W., 2007. An investigation of inbreeding depression and purging in captive pedigreed populations. *Heredity*, 98, 172-182.

Burger, J., Rosendahl, W., Loreille, O., Hemmer, H., Eriksson, T., Gotherstrom, A., Hiller, J., Collins, M. J., Wess, T., Alt, K.W., 2004. Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*. *Molecular Phylogenetics and Evolution*, 30, 841-849.

Dakin, E.E., Avise, J. C., 2004. Microsatellite null alleles in parentage analysis. *Heredity*, 93, 504-509.

## Genetic diversity in a captive population of Asian lions

Dorman, N., 2009. European Studbook for the Asiatic Lion (*Panthera leo persica*) Number 5 (2007-2009).

Driscoll, C.A., Menotti-Raymond, M., Nelson, G., Goldstein, D., O'Brien, S.J., 2002. Genomic microsatellites as evolutionary chronometers: A test in wild cats. *Genome Research*, 12, 414-423.

Dubach, J., Patterson, B.D., Briggs, M.B., Venzke, K., Flamand, J., Stander, P., Scheepers, L., Kays, R.W., 2005. Molecular genetic variation across the southern and eastern geographic ranges of the African lion, *Panthera leo*. *Conservation Genetics*, 6, 15-24.

Frankham, R., Ballou, J., Briscoe, D.A., 2010. *Introduction to Conservation Genetics*, 2nd ed. Cambridge University Press, Cambridge.

Gaur, A., Shailaja, K., Singh, A., Arunabala, V., Satyarebala, B., Singh, L., 2006. Twenty polymorphic microsatellite markers in the Asiatic lion (*Panthera leo persica*). *Conservation Genetics*, 7, 1005-1008.

Gautschi, B., Jacob, G., Negro, J.J., Godoy, J.A., Muller, J.P., Schmid, B., 2003. Analysis of relatedness and determination of the source of founders in the captive bearded vulture, *Gypaetus barbatus*, population. *Conservation Genetics*, 4, 479-490.

Gilbert, D.A., Packer, C., Pusey, A.E., Stephens, J.C., O'Brien, S.J., 1991. Analytical DNA Fingerprinting in lions - Parentage, genetic diversity, and kinship. *Journal of Heredity*, 82, 378-386.

## Genetic diversity in a captive population of Asian lions

Goldstein, P.Z., Desalle, R., Amato, G., Vogler, A.P., 2000. Conservation genetics at the species boundary. *Conservation Biology*, 14, 120-131.

Goncalves Da Silva, A., Lalonde, D.R., Quse, V., Shoemaker, A., Russello, M.A., 2010. Genetic approaches refine ex situ lowland tapir (*Tapirus terrestris*) conservation. *Journal of Heredity*, 101, 581-590.

Guardian (2015). India's Asiatic lion population rising. Available at <http://www.theguardian.com/environment/2015/may/11/indias-asiatic-lion-population-rising> [accessed on 3rd November 2015] [Online].

Ivy, J.A., Miller, A., Lacy, R.C., Dewoody, J.A., 2009. Methods and prospects for using molecular data in captive breeding programs: An empirical example using parma wallabies (*Macropus parma*). *Journal of Heredity*, 100, 441-454.

Kalahari Predator Conservation Trust, 2013. Lions. Available at <http://www.kalahari-predator-conservation.com/en/predators/lions> [accessed on 23rd August 2012] [Online].

Kitchener, A.C., Breitenmoser-Wursten, Ch., Eizirik, E., Gentry, A., Werdelin, L., Wilting, A., Yamaguchi, N., Abramov, A.V., Christiansen, P., Driscoll, C., Duckworth, J.W., Johnson, W., Luo, S.-J., Meijaard, E., O, Meijaar, P., Sanderson, J., Seymour, K., Bruford, M., Groves, C., Hoffmann, M., Nowell, K., Timmons, Z., Tobe, S., 2017. *A revised taxonomy of the Felidae*. The final report of the Cat Classification Task Force of the IUCN/SSC Cat Specialist Group. *Cat News Special Issue* 11, pp.1-80.

## Genetic diversity in a captive population of Asian lions

Lacy, R. C., Ballou, J. D., Pollak, J. P., 2012. PMx: software package for demographic and genetic analysis and management of pedigreed populations. *Methods in Ecology and Evolution*, 3, 433–437.

Miquel, C., Bellemain, E., Poillot, C., Bessière, J., Durand, A., Taberlet, P., 2006. Quality indexes to assess the reliability of genotypes in studies using noninvasive sampling and multiple-tube approach. *Molecular Ecology Notes*, 6, 985-988.

O'Brien, S.J., 1994. A role for molecular genetics in biological conservation. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 5748-5755.

O'Brien, S.J., Martenson, J.S., Packer, C., Herbst, L., De Vos, V., Joslin, P., Ott-Joslin, J., Wildt, D.E., Bush, M., 1987. Biochemical genetic variation in geographic isolates of African and Asiatic lions. *National Geographic Research*, 3, 114-124.

Paulson, M., 1999. Pride in place: Reintroduction of Asiatic Lions in Gir Forest, India. *Restoration and Reclamation Review*, 4.

Raymond, M., Rousset, F., 1995. Genepop (Version-1.2) - Population-genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248-249.

Rousset, F., 2008. GENEPOP '007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8, 103-106.

Russello, M.A., Amato, G. 2004., Ex situ population management in the absence of pedigree information. *Molecular Ecology*, 13, 2829-2840.

Genetic diversity in a captive population of Asian lions

Ryder, O.A., 1986. Species conservation and systematics - the dilemma of subspecies. Trends in Ecology & Evolution, 1, 9-10.

Singh, A., Shailaja, K., Gaur, A., Singh, L., 2002. Development and characterization of novel microsatellite markers in the Asiatic lion (*Panthera leo persica*). Molecular Ecology Notes, 2, 542-543.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L. P., Bouvet, J., 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research, 24, 3189-3194

## Genetic diversity in a captive population of Asian lions

Table 1: Details of the original nine founders of the European captive population of the Asian lion in the collections of National Museums Scotland (NMS) and the Finnish Museum of Natural History (FMNH).

EAZA studbook no.	Sex	Date of birth	Place of birth	Zoo	Zoo accession no.	Date of death	Museum	Museum register no.	Tissue	Name
1181	F	6.12.88	Sakkarbaugh Zoo	Zurich	910052	10.2.05	NMS	Z.2015.86.2	muscle	Mena
1187	M	3.4.89	Sakkarbaugh Zoo	Zurich	910053	28.3.08	NMS	Z.2015.86.1	muscle	Bhagirath
1195	F	2.5.89	Sakkarbaugh Zoo	Chester	24889	10.5.05	NMS	Z.2009.8	muscle	Chandani
1196	F	2.5.89	Sakkarbaugh Zoo	London	A1272	9.2.07	NMS	Z.2007.15	muscle	Ruchi
1214	F	6.3.91	Sakkarbaugh Zoo	Helsinki	920203	8.11.00	FMNH	UN 2042	skin/bone	Leslie
1217	F	20.3.91	Sakkarbaugh Zoo	Helsinki	920202	16.8.03	FMNH	UN 2080	skin/bone	Kirtida
1235	M	5.7.88	Gir Forest	Boissière	FLA3	2.9.06	NMS	Z.2007.10	muscle	Vanaraj
1245	M	c.1989	Gir Forest	Boissière	FLA1	5.12.00	NMS	Z.2002.185.1	bone	Jake
1253	M	c.1989	Gir Forest	London	A1270	9.1.95	NMS	Z.1995.5	bone	Arthur

## Genetic diversity in a captive population of Asian lions

Table 2. Genotyping summary for the EEP founders in comparison to data from the Indian population. (N= number of individuals, K= number of observed alleles, H<sub>O</sub>= Observed heterozygosity, H<sub>E</sub>= Expected heterozygosity. \*marker failed to amplify in some individuals \*\*one individual amplified two alleles in some repeated tests (62/7 tests) and showed allelic drop-out of one or the other allele in the other repeated tests.

		Marker (Ple)											
		23	24	51	55	57	21	34	53	58	62	65	251
EEP Founders	N	9	9	9	9	9	8*	7*	9	8*	9	9	9
	K	1	2**	1	2	2	1	1	1	1	1	1	1
	H <sub>O</sub>	-	0.11	-	0.78	0.33	-	-	-	-	-	-	-
	H <sub>E</sub>	-	0.11	-	0.48	0.50	-	-	-	-	-	-	-
Indian population	N	15	15	15	15	15	22	22	22	22	22	22	22
	K	8	6	8	11	7	6	5	6	4	6	5	4
	H <sub>O</sub>	1.00	0.06	0.86	0.86	1.00	0.95	0.57	0.94	0.63	0.68	0.84	0.84
	H <sub>E</sub>	0.58	0.41	0.85	0.71	0.58	0.68	0.61	0.77	0.62	0.77	0.75	0.60

## Genetic diversity in a captive population of Asian lions

## Figures

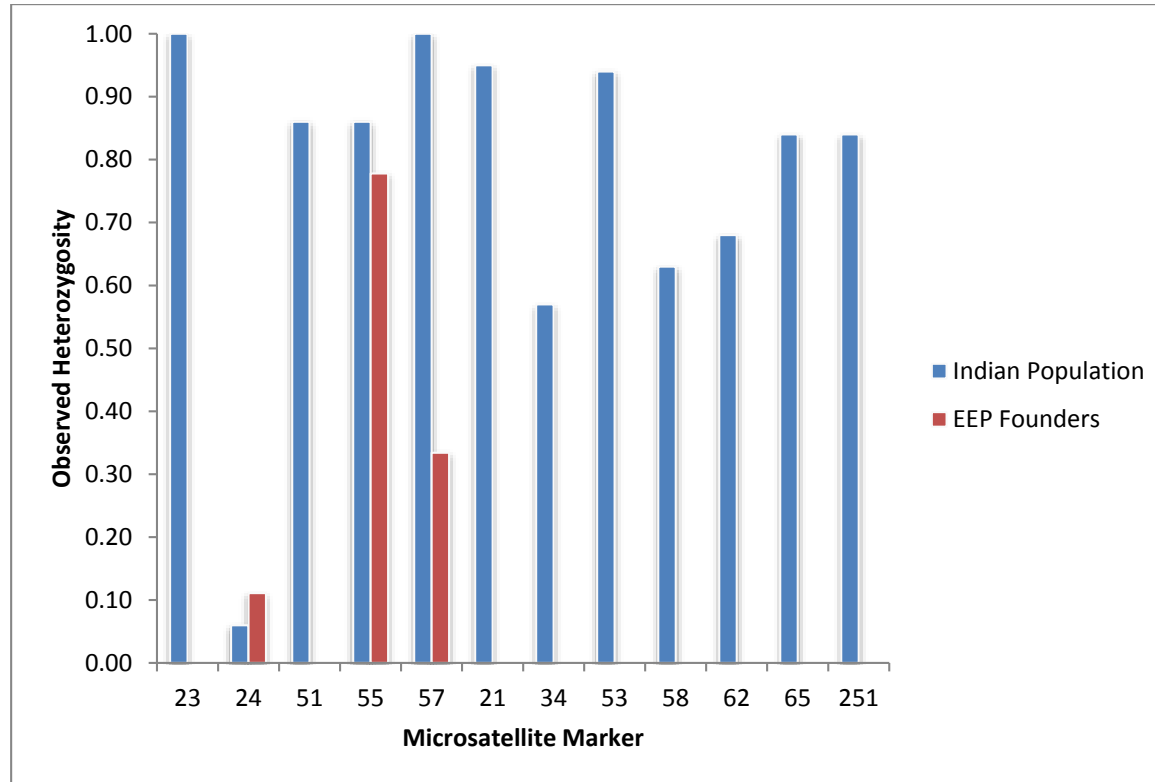


Figure 1. Comparison of observed heterozygosity values for the 12 markers utilised in this study. EEP Asian lion founders are represented by red bars and current Indian captive populations are represented by blue bars.



## Genetic diversity in a captive population of Asian lions

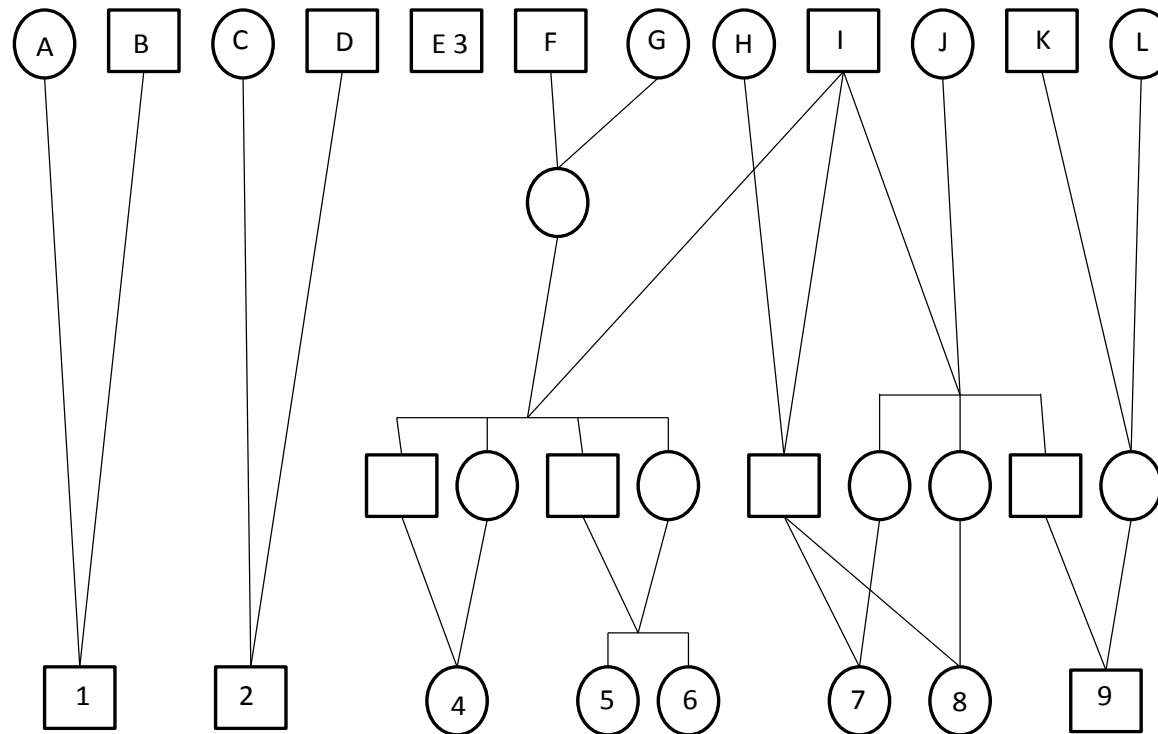


Figure 2. Schematic to show pedigree of EEP Asian lion founders (1-9) in relation to wild-caught ancestors (A-L). Males are symbolised by squares; females by circles. The male labelled 'E 3' is considered both a wild-caught ancestor, and an EEP founder. Individuals labelled 4-9 can all be traced to one common grandfather (I) and individuals 4-8 are the offspring of full-sibling or half-sibling matings